Exploiting semiochemicals in insect control†

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Abstract: The present situation and future prospects for the use of semiochemicals for insect control is reviewed, with particular reference to the work being carried out at IACR-Rothamsted. The techniques used to identify pheromones and other semiochemicals, and the types of compound found in various insect classes, are described. The effects of such compounds on pests, their predators and other members of the ecosystem are considered in relation to the development of control strategies such as 'push–pull' or stimulo-deterrent diversionary strategies (SDDS).

Keywords: semiochemicals; insect control; SDDS

1 INTRODUCTION

Semiochemicals (from the Greek *σημειον*, semeion, meaning sign or signal) are chemicals mediating interactions between organisms,[1] either within the same species (pheromones) or from different species (allelochemicals).[2] A semiochemical may influence interactions involving a number of organisms from several trophic levels. Bark beetles, for example, aggregate on trees using semiochemicals produced by conspecific beetles, the attraction of which is synergised by volatiles released from the tree itself.[3] The same compounds may attract other insects utilising the tree for food or oviposition, inhibit the development of fungi or bacteria and may also have a role in plant/plant interactions. A complex naming system has evolved to classify semiochemicals depending on the benefits or detriments resulting from the interaction (eg kairomone, allomone, synomone)[2,4] but in this review, only the terms semiochemical, pheromone and allelochemical will be used.

The study of semiochemicals, and the interactions they mediate, is part of chemical ecology and contributes to an understanding of the behaviour, development and evolution of organisms. However, from a practical point of view, such research also provides the basis for successful use of semiochemicals for pest control as an alternative to exclusive use of broad-spectrum toxicants. Insects use chemical information from their environment at all stages of development, to locate food, oviposition and hibernation sites, to come together with conspecifics and sexual partners, and to avoid dangerous situations or unsuitable habitats and hosts. Semiochemicals that have the ability to attract or repel insects, or that enhance (synergise) or inhibit the action of other chemicals, have the potential to be used in direct control of pests by mass trapping or mating disruption, or in deterring pests from food and oviposition sites.[5–7] Semiochemicals, being involved in multitrophic interactions, can also be used to influence the behaviour of natural enemies of pests. Some or all of these activities can be utilised as components of integrated pest control strategies.

2 STRATEGIES FOR USE OF SEMIOCHEMICALS IN PEST CONTROL

The semiochemicals that have been used most successfully in pest control are lepidopterous sex pheromones and the aggregation pheromones of Coleoptera.[8,9] Many commercially developed systems exist for use of lepidopterous sex pheromones, either in monitoring systems or in slow-release formulations to disrupt normal mate location. For control of forest pests, aggregation pheromones of bark beetles are used in trap-out procedures. However, semiochemicals, when employed alone, may give ineffective or insufficiently robust pest control and, particularly with the main pest of arable agriculture in Northern Europe, alternative approaches must be considered. This review will

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concentrate on aspects studied at IACR-Rothamsted and in collaborative projects.

Semiochemicals will, in the future, find use within push-pull or stimulo-deterrent diversionary strategies (SDDS).[10] In such approaches (Fig 1), the harvestable crop is protected by means of semiochemicals such as plant-derived antifeedants, by employing repellent crop cultivars and by exploiting semiochemicals from non-host plants which interfere with location of the host plant by the pest. Aggregation of pests away from the crop is encouraged by attractants such as sex and oviposition pheromones and by trap crops producing large quantities of host attractants. The trap crop can also be treated with a population-reducing component such as a highly selective pesticide, or a pathogenic biological control agent for which conditions on the trap crop can be modified to benefit its development. These semiochemically based control strategies should also be designed to exploit natural populations of beneficial insects such as predators and parasitoids. Each component of the SDDS, when compared with conventional broad-spectrum toxicants, is relatively ineffective; this has the advantage of not selecting strongly for resistance and thus contributes to the overall sustainability of the approach.

2.1 Isolation and identification of semiochemicals

The process of semiochemical isolation and identification can be exemplified for a phytophagous insect colonising an arable crop. The first step is to locate the sources providing volatile cues for the insect and, by using behavioural studies, to answer such questions as: is the insect attracted to intact host plants or to plants already damaged by other insects?; does the insect prefer to attack a specific growth stage or specific parts of the plant?; is the insect attracted over a short or long distance?; are other organisms associated with the host plant providing additional semiochemical-based information? Volatile semiochemicals mediating such interactions can be isolated using techniques of air entrainment and headspace analysis.[11,12] The semiochemical source is enclosed in a clean glass chamber and the volatile chemicals released by the sample are swept through a polymer trap in a stream of purified air. The trapped chemicals can be eluted from the polymer using a solvent, providing a liquid extract for further investigations. Many of the compounds present in the extract are of no relevance to the insect, so to identify all the components, and to evaluate individually their ability to elicit behavioural responses, would be a long and tedious procedure. This process can be made more efficient by using electrophysiological recording techniques.

'PUSH'	'PULL'
(Away from the crop)	(Into traps or trap crops)
Masking of host attraction	Host attractants
Repellents, antifeedants, oviposition deterrents	Aggregation, sex and oviposition pheromones
Attractants for predators and parasitoids	Visual cues
	Selective control agents (e.g. pathogens)

Figure 1. Components of the Push-Pull or Stimulo-Deterrent Diversionary Strategy.

Insects perceive volatile semiochemicals via olfactory receptors (sensory cells) on the antenna which, when stimulated, pass information to the brain in the form of an electrical signal, or action potential, which can be measured.[13] Electrophysiological activity can be assessed either by electroantennography (EAG), in which, by placing an electrode at each end of the antenna, the overall responses of the olfactory cells can be measured, or by recording from individual olfactory receptors within the sensilla (single cell recording, or SCR). By linking this system with high-resolution gas chromatography (GC), ie splitting the effluent from the GC column and presenting it simultaneously to the flame ionisation detector (FID) of the GC and the antennal preparation, it is possible to locate compounds within a complex extract which have biological activity.[14] Such a system is shown diagrammatically in Fig 2, with a typical coupled trace in Fig 3. Active compounds are identified using coupled gas chromatography-mass spectroscopy (GC-MS)[15] and confirmed by co-injection on GC with authentic compounds.

Electrophysiological activity for a compound, although suggesting that the material is of importance to the insect, gives no indication of its behavioural role, nor whether it is active only at a particular concentration or only in combination with other components. Other signals, including visual cues, may also be necessary for behavioural activity. Identified compounds therefore require assessment in laboratory bioassays such as olfactometer or wind-tunnel studies, using insects at the correct physiological stage to elicit the behaviour of interest. Promising compounds, or mixtures of compounds, can then be tested in the field for their ability to influence behaviour of naturally occurring populations of the pest and its natural enemies. If field trials are successful, the process of formulating, applying and commercialising the compounds can then be initiated.[16]

2.2 Components of integrated control strategies

2.2.1 *Host location*

For phytophagous insects, plant volatiles play an important role in host location. Investigations into the chemical ecology of coleopterous pests of oilseed rape (*Brassica napus* L.), eg the seed weevil, *Ceutorhynchus assimilis* (Payk)[17] and the cabbage stem flea beetle, *Psylliodes chrysocephala* L, have identified

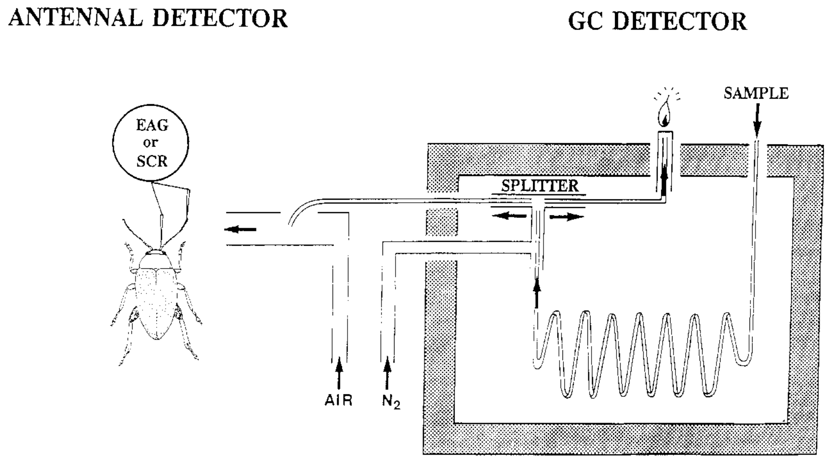

Figure 2. The coupled GC-electrophysiology system.

as many as 25 oilseed rape volatiles which are electrophysiologically active at the relative levels released by the plant. These include isoprenoids and compounds derived from amino and fatty acids. Whilst most of the compounds are ubiquitous plant volatiles, the isothiocyanates, which are catabolites of glucosinolates, are characteristic of the Brassicaceae. For unadapted organisms, the isothiocyanates can be poisonous or repellent, but for insects adapted to brassicas they act as attractants and phagostimulants. Thus, in field trials within an oilseed rape crop, water traps baited with these compounds, particularly the 2-phenylethyl, 3-butenyl and 4-pentenyl isothiocyanates, caught significantly more *C assimilis* than unbaited traps.[18]

Concern over the environmental effects of pesticides and the economics of their usage is providing increased interest in obtaining accurate population estimates of spring and summer pests of oilseed rape. Populations of these pests, particularly *C. assimilis* and the pollen beetles, *Meligethes* spp, are currently assessed by the U K Agricultural Development and Advisory Service (ADAS) by tray beating, which has considerable drawbacks. Yellow sticky traps with isothiocyanate lures are now being evaluated as a more reliable and practical monitoring system; results suggest that trap data collected in early spring can provide advance warning of the main migration of oilseed rape pests into the crop.[19]

Electrophysiological studies have revealed that perception of plant volatiles, even those which are ubiquitous within the plant kingdom, is mediated by specific olfactory cells. For *C assimilis*, it was also observed that a number of compounds, from different biosynthetic pathways, are detected by olfactory cells which frequently occur in pairs,[17] which may allow the insect to assess accurately the ratios of compounds being released.

Western flower thrips (WFT), *Frankliniella occidentalis* (Perg), are an important pest of ornamentals

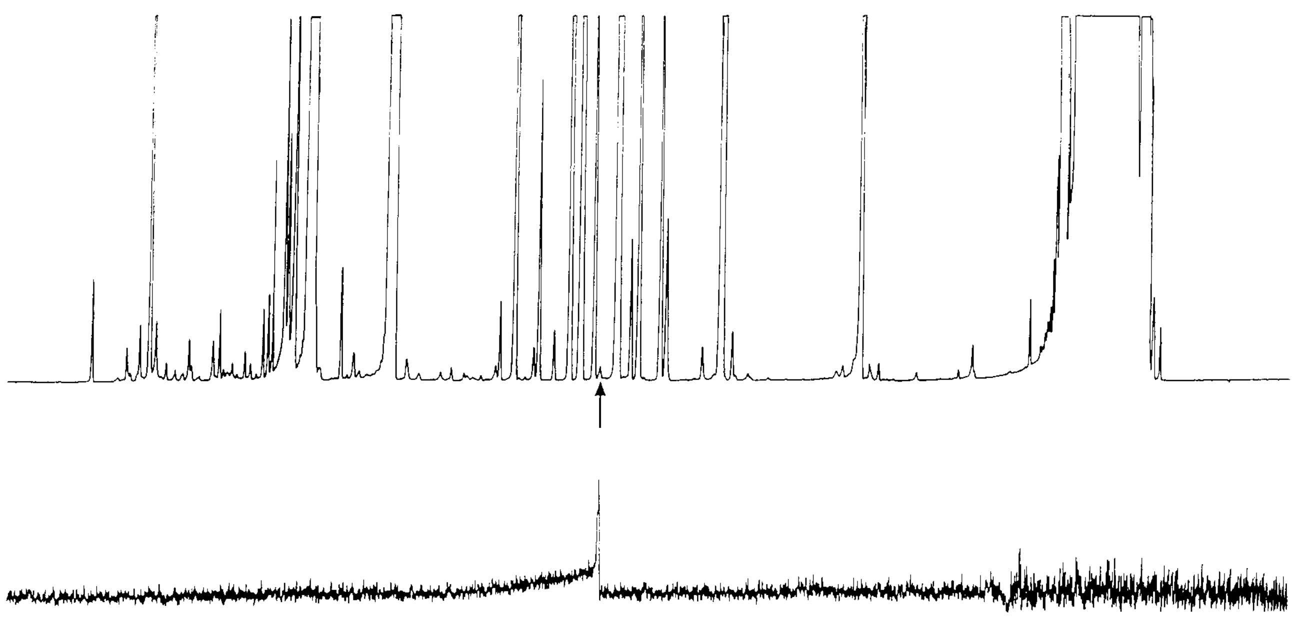

Figure 3. Coupled GC-single cell recording with raspberry beetle, *Byturus tomentosus*. Upper trace: GC of volatiles from fennel, *Foeniculum vulgare;* lower trace: simultaneous response of olfactory cell (C M Woodcock, unpublished data).

and vegetables grown under glass. Little is known about semiochemicals mediating host location by these insects, although attraction to flower volatiles has been demonstrated in field experiments. In the olfactometer, bud stages of chrysanthemums were more attractive to WFT adults than were leaves or fully open flowers.[20] Volatiles from infested and uninfested buds were collected by air entrainment, and active components were located by coupled GC-EAG and identified by GC-MS.[21] Whilst chrysanthenone was the major component in both samples, levels of some components, particularly sesquiterpene hydrocarbons including β-caryophyllene, (*E*)-β-farnesene, germacrene-D and (*E*,*E*)-α-farnesene, were higher in the infested buds. (*E*)-β-Farnesene, one of the electrophysiologically active components associated with infested buds, was attractive to WFT in the olfactometer and, in glasshouse trials, more thrips were caught on blue sticky traps baited with this compound than on unbaited controls. This is currently being evaluated as a monitoring system for WFT.

The increasing level of pesticide resistance in thrips is placing greater emphasis on the development of alternative control measures. Although integrated pest management strategies involving the use of predatory bugs and mites are used for control of WFT in glasshouses, these can be expensive. In a collaborative programme with ADAS, the use of trap plants is being investigated to attract WFT from the main crop and concentrate them in sites where control agents can be deployed, thus minimising labour costs.[22] The approach aims to exploit the differential attraction of WFT to vegetative and opening flowers observed with chrysanthemums. In olfactometer studies, flowers from *Verbena x hybrida* Voss, particularly the cultivars Sissinghurst, Tapien Pink and Pink Parfait, were highly attractive to WFT, suggesting their potential as trap plants. In glasshouse trials, these flowering plants were highly effective in attracting WFT away from crops of ivy-leaf geraniums and chrysanthemums. The main volatile components from Tapien Pink and Pink Parfait were collected by air entrainment and identified by GC-MS and nuclear magnetic resonance as the linalool oxide pyrans **1** and **2** (Fig 4). Enantioselective synthesis using Sharpless asymmetric dihydroxylation methodology and analysis by chiral GC enabled verification of the absolute stereochemistry. In the olfactometer, **1** was attractive to WFT whilst no attraction was observed with either **2** or a mixture of the two, suggesting that activity is, at least in part, associated with the presence of additional compounds. Indeed, a number of other compounds have been identified in these volatiles, including (*E*)-4,8-dimethyl-1,3,7-nonatriene (**3**) and the tetraene **4** (Fig 4). The former compound is of particular interest, since it has already been implicated as a semiochemical mediating attraction of beneficial insects to herbivore-damaged plants.[23]

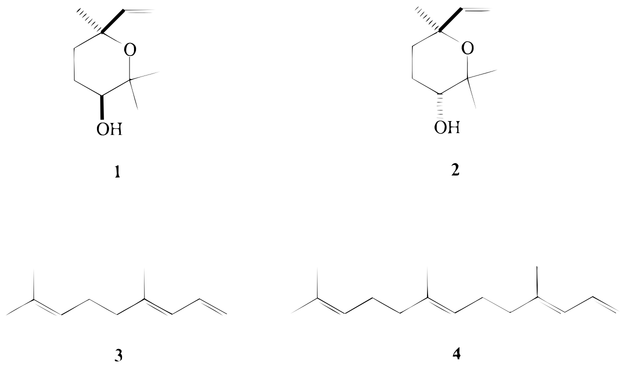

Figure 4. Volatiles identified in *Verbena x hybrida*: **1** and **2** linalool oxide pyrans; **3** (*E*)-4,8-dimethyl-1,3,7-nonatriene; **4** (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene.

2.2.2 Non-host recognition and avoidance

It had been assumed that, for many insects, avoidance of non-hosts is based on initial attempts at colonisation or feeding which are terminated by lack of appropriate physiology, nutritional aspects or by the detection of potentially toxic secondary metabolites. However, new work has demonstrated that an earlier stage of non-host avoidance exists in which insects can detect, at a distance, volatile metabolites as cues indicating acceptability or unsuitability of the host.

Many aphids such as the black bean aphid, *Aphis fabae* Scop, and the peach-potato aphid, *Myzus persicae* (Sulz), are able to exploit a wide range of host plants. However, although *A fabae* does not feed on plants in the Brassicaceae, electrophysiological studies have shown that it possesses olfactory cells tuned to the detection of isothiocyanates, the volatile catabolites of glucosinolates characteristic of this family. Indeed, the response profiles of such cells in *A fabae* are almost identical to those found in the brassica specialist *Brevicoryne brassicae* (L), the cabbage aphid.[24] *A fabae* also avoids landing on many highly aromatic plants, particularly those in the Lamiaceae; as with the brassicas, it was shown that a compound typical of the family, and also of the resin-producing gymnosperms, (1*R*,5*S*)-myrtenal (**5**, Fig 5), is detected by highly specific olfactory cells. In olfactometer studies, the isothiocyanates and (1*R*, 5*S*)-myrtenal were repellent to *A fabae* and interfered with the normal attraction to the aphid's host plants.[24,25] Thus, a mechanism is demonstrated whereby an insect feeding mainly on the Fabaceae

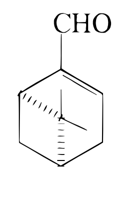

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Figure 5. (1*R*,5*S*)-Myrtenal, a repellent and host-masking agent for *Aphis fabae*.

could avoid a wide range of non-host plants, and ecosystems such as conifer forests where host plants are unlikely to be present. The principle of non-host recognition mediated by olfactory perception of specific volatile cues provides a new aspect to the development of semiochemically mediated pest control strategies and further studies have been conducted to investigate this approach in more detail.

The bird cherry-oat aphid, *Rhopalosiphum padi* (L), colonises as its primary or winter host the bird cherry tree, *Prunus padus* L. In the spring, alate (winged) forms are produced which migrate into cereal crops, the herbaceous summer (secondary) host. It was suggested that, whilst volatiles from the winter host may be attractive for the autumn migrants, spring migrants may find such compounds repellent. By using coupled GC-SCR on spring migrants of *R. padi* with volatiles from the winter host *P. padus*, strong electrophysiological activity was found for a minor component which was identified as methyl salicylate and, in olfactometer assays, this compound significantly reduced attraction to cereal leaves.[26] Field trials with methyl salicylate in the UK and Sweden reduced colonisation of cereal crops by *R padi* and other cereal aphids by approximately 50%. Models predict that this level would be sufficient to allow natural enemies such as predators and parasitoids to reduce aphid populations to below acceptable damage levels.[27]

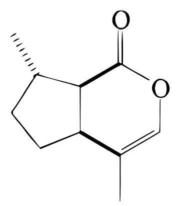
The activity of methyl salicylate for cereal aphids other than *R. padi*, particularly the grain aphid, *Sitobion avenae* (F), which does not host-alternate, suggests that the role of this compound may be based more in the plant defence system than in the plant being taxonomically an inappropriate host. Indeed, this compound has been the subject of an investigation covering over 30 species of insects from four Orders, and all species tested showed some electrophysiological response (CM Woodcock, unpublished data). The phenylalanine ammonia lyase pathway in plants is induced on herbivory and pathogen development. One compound involved in signalling associated with this pathway is salicylic acid, and methyl salicylate, due to its volatility, could act externally as a related signal. Indeed, this has now been demonstrated for the induction of plant defences against pathogens, where methyl salicylate acts as a volatile semiochemical for the plant by inducing endogenous defence systems.[28]

2.2.3 Pheromones

Aphids, when attacked by predators, release an alarm pheromone causing dispersal of other aphids in the immediate area. For most species, the major component of the alarm pheromone is the sequiterpene hydrocarbon (*E*)-*β*-farnesene (see Ref 29 and references therein). Attempts have been made to use the pheromone to increase aphid mobility and thereby improve pick-up of control agents such as fungal pathogens or contact pesticides. With the cotton aphid, *Aphis gossypii* Glov, on glasshouse ornamental crops, although the pheromone gave only a small increase in mobility, this was sufficient to increase pick-up of spores of the fungal pathogen *Verticillium lecanii* (Zimm) Viégas and produce acceptable mortality for pest management.[30] Similar results were obtained in the field, combining the alarm pheromone with contact pesticides such as the pyrethroid permethrin. In both cases, an electrostatic spraying system was used to present the alarm pheromone to the aphids in the most efficaceous manner.[31]

Depending on species and climate, many aphids feed and reproduce parthenogenetically (asexually) throughout the summer on their herbaceous secondary hosts. In the autumn, pre-sexual female forms (gynoparae) migrate to the primary or winter hosts where true sexual females (oviparae) are produced. On maturity, these individuals release, from scent plaques on the hind tibiae, a sex pheromone which is used by males to locate their mates (see Ref 29 and references therein). Coupled GC-SCR on the male antenna was used to locate active components in the volatiles released by oviparae. For many species of aphids, the sex pheromones were found to comprise one or both of the nepetalactone **6** and the nepetalactol **7** (Fig 6), in varying ratios.[32] Each compound is detected by a particular olfactory cell type and dose–response data showed high specificity of these cells for the respective compounds.[33] In contrast, the sex pheromone of the damson-hop aphid, *Phorodon humuli* (Schr), comprises two diastereomers of the nepetalactol **8** (**a** and **b**, Fig 6).[34]

In autumn field trials at Imperial College, Silwood Park, using water traps releasing compounds **6** and **7**, males of different species were selectively trapped.[35] These traps can be highly effective. In collaborative studies in Korea, catches of over 1000 males per day were achieved for a number of aphid species including *Tuberocephalus momonis* (Matsumura), *Lachnus tropicalis* (v.d. Goot) and *Aphis citricola* v.d. Goot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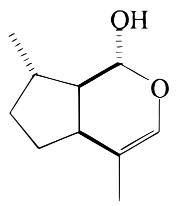
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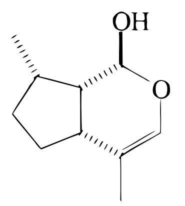
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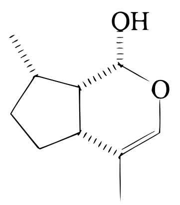
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Figure 6. Aphid sex pheromone components.

(K S Boo, M Y Choi, V F Eastop, I B Inbum, J A Pickett, L J Wadhams & C M Woodcock, in preparation).

Similarly, in an autumn field trial in the UK, six water traps containing the *P humuli* sex pheromone caught over 3000 males, whereas a suction trap sampling over $500\,m^3\,h^{-1}$ of air caught less than 400. Male aphids were also observed to orientate towards the pheromone traps against surprisingly strong winds.[36] Although these studies have led to commercial consideration of mating disruption and trap-out as possible control measures for *P humuli*, collaborative efforts between IACR-Rothamsted and Horticulture Research International, East Malling, are using pheromones to attract males into traps containing a particular strain of the fungal pathogen *V lecanii* which is effective at the low field temperatures occuring during the autumn migration.

Many insects require compounds from the host plant to maximise pheromone attraction. Indeed, in initial trials with the sex pheromone of *P. humuli*, volatiles from the primary host, *Prunus* spp, synergised the attraction of males; a similar effect was noted for *R padi* with volatiles from *P padus*.[37] The pea and bean weevil, *Sitona lineatus* L, a significant pest in Europe, the USA and the Middle East, is polyphagous on many wild and cultivated members of the Fabaceae, but optimum reproduction occurs only on peas (*Pisum sativum* L), beans (*Vicia faba* L) and vetches (*Vicia sativa* L). The aggregation pheromone of *S. lineatus* was identified as the 1,3-diketone, 4-methyl-3,5-heptanedione (**9**, Fig 7),[38] and GC-EAG analysis of volatiles from beans located a number of electrophysiologically active components subsequently identified as (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate and linalool. Although these volatiles are ubiquitous in the plant kingdom, they were shown to synergise attraction to the aggregation pheromone and provided a highly effective lure for trapping these weevils in the field.

The synthetic *S. lineatus* aggregation pheromone is being used in cone traps to monitor the activity of weevils on overwintering sites in the early spring.[39] Such a system can be used to indicate imminent migration of the weevil from the overwintering sites into the crop and hence to allow accurate timing of insecticide application. More importantly, it could also be used to show that pesticide application is unnecessary if weevil numbers are low, or if the crop is not at a susceptible growth stage at the time of *S lineatus* migration from the overwintering sites. This monitoring system is currently under evaluation by growers.

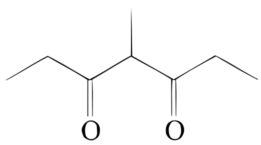

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Figure 7. *Sitona lineatus* aggregation pheromone.

2.2.4 *Beneficial insects*

Plant volatiles play an important role in host location, not only by phytophagous insect pests, but also by their natural enemies, and the functions of such semiochemicals in tritrophic interactions are receiving increased attention.[40] Herbivore feeding damage stimulates plants to release significantly increased quantities of volatiles which are attractive to predators or parasitoids, a process mediated by systemic interactions that serve as a plant defence mechanism.[41,42]

Herbivore-induced release of plant volatiles mediating the foraging behaviour of hymenopterous aphid parasitoids, particularly *Aphidius ervi* Haliday, has been demonstrated with a host/plant complex comprising the pea aphid, *Acyrthosiphon pisum* (Harr), on beans, *V faba*.[43] GC-EAG studies on volatiles from beans infested with *A pisum* located a number of peaks showing activity for *A ervi* females, and the identified compounds were investigated in wind-tunnel assays. Of the compounds tested, 6-methyl-5-hepten-2-one was the most attractive for *A. ervi* females, with linalool, (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene, (*Z*)-3-hexen-1-ol and (*E*)-β-farnesene also eliciting significant oriented flight behaviour. Foraging experience significantly increased parasitoid response to these compounds, with the exception of (*E*)-β-farnesene. Feeding of *A pisum* on *V faba* induced or increased the release of several compounds, including 6-methyl-5-hepten-2-one, and comparative analyses of the volatiles from beans infested with either *A pisum* or the non-host aphid *A fabae* revealed that this compound is not produced by the latter. 6-Methyl-5-hepten-2-one is therefore one of the volatile components that may allow *A ervi* to distinguish between plants colonised by a host or non-host aphid species.

During field trials with the aphid sex pheromones, it was observed that females of certain aphid parasitoids were also caught in traps baited with the pheromone components.[44] In subsequent trials, increased parasitism of *S avenae* by the parasitoid *Praon volucre* (Haliday) was observed in plots treated with the nepetalactone **6**.[45] However, for certain crops, it would be preferable to attract other aphid parasitoids, for example *A ervi* against *A pisum*, and EAG studies have demonstrated that a number of species, including *A ervi*, possess receptors for the aphid sex pheromone components.[46] *A pisum* employs both the nepetalactone **6** (Fig 6) and the nepetalactol **7**, in a 1 : 1 ratio, as its sex pheromone; by using lures with this formulation in a field trial using potted plants, parasitism of *A pisum* by *A ervi* was increased by more than 300%.[47]

Whilst the aphid sex pheromones are produced naturally only in the autumn, aphid parasitoids will respond to these stimuli throughout the year.[48] In

addition, the response to these compounds is innate, ie not learned, as is the situation with some semiochemicals associated with the host/plant complex, thus providing new opportunities for the manipulation of natural populations of aphid parasitoids.[40] This work has now formed the basis for commercially funded programmes in which hedgerows, or set-aside strips within the crop, are used to build up parasitoid populations so that foraging behaviour can be stimulated in neighbouring cereal crops by means of the aphid sex pheromones.

Based on successes in manipulating populations of parasitoids, work has now been directed towards other beneficial insects, particularly the more polyphagous predators. In collaborative studies in Korea, the response of the lacewing *Chrysopa cognata* Wesmael, one of the principal aphid predators, to aphid pheromones was investigated.[49] EAG responses were obtained for the sex pheromone components but not the alarm pheromone (*E*)-*β*-farnesene. Similarly, in laboratory and field studies, the sex pheromone components were attractive to *C cognata*, whilst no attraction was observed to (*E*)-*β*-farnesene.

The seven-spot ladybird, *Coccinella septempunctata* L., is an important coleopterous predator aiding the biological control of aphids. However, little is known of the ecology of this species, particularly the mechanisms by which its prey is located, the intraspecific aggregation that occurs prior to overwintering, or the impact on predation of its own natural enemies. Laboratory behavioural studies in collaboration with the University of Uppsala, Sweden, showed that overwintering adult *C septempunctata* were attracted to volatiles from conspecific adults. The active compound was identified as 2-isopropyl-3-methoxypyrazine (**10**, Fig 8) which was also shown, by coupled GC-organoleptic (olfactory) evaluation of an extract of *C. septempunctata* volatiles, to be responsible for the odour regarded by human beings as being characteristic of ladybirds.[50]

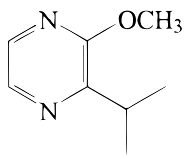
Electrophysiological studies have shown that the *C. septempunctata* antenna possesses specific, and often paired, olfactory cells for the detection of *β*-caryophyllene and (*E*)-*β*-farnesene, and in the olfactometer adult *C. septempunctata* were attracted to both compounds.[51] In further studies on the chemical ecology of *C septempunctata*, it was demonstrated that the ladybird parasitoid *Dinocampus coccinellae* Schrank was attracted to *C septempunctata* volatiles; the active compound was identified by coupled GC-EAG and GC-MS as the defence alkaloid precoccinelline (**11**, Fig 8).[51] The potential for manipulating ladybird and parasitoid populations with these semiochemicals is currently being investigated in the field.

2.3 Semiochemicals of haematophagous pests

2.3.1 *Mosquitoes*

Mosquitoes in the genus *Culex*, and in particular *Culex quinquefasciatus* Say, lay rafts of eggs which, on maturation, develop droplets at their apices releasing a pheromone attractive to other gravid females. The major component was identified as (5*R*,6*S*)-acetoxy-5-hexadecanolide (**12**, Fig 9) (see Ref 52 and references therein). Field trials in Kenya, conducted at the International Centre for Insect Physiology and Ecology (ICIPE) at Mbita Point, showed the pheromone to be highly effective in eliciting oviposition, giving an 80% increase in numbers of egg rafts laid in the pheromone treatments compared to the controls. To kill the larvae ensuing from increased oviposition, a selective insect growth regulant, pyriproxifen, which has a mode of action related to that of the juvenile hormones, was included in the pheromone formulation. Subsequently, the pheromone has been tested successfully in a number of widely differing geographical areas, most recently in Tanzania.[53] These studies also revealed that cues associated with the oviposition site were essential for optimal attraction.

Culex spp mosquitoes employ volatile semiochemicals, together with visual cues, to locate oviposition sites and a number of active compounds, including 3-methyl indole, have been identified. Studies with natural oviposition pheromone and with racemic synthetic material (see Ref 52) have demonstrated the essential nature of oviposition-site semiochemicals in optimising activity of the pheromone. Indeed, trials conducted in various geographic locations indicated a wide range of sources for such semiochemicals. In laboratory studies, water polluted by various materials, including faeces of rabbit, *Oryctolagus cuniculus* L, gave an additive effect with the oviposition pheromone. GC-SCR with samples of



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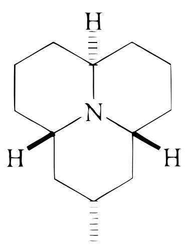

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Figure 8. Semiochemicals from *Coccinella septempunctata*: **10** 2-isopropyl-3-methoxypyrazine; **11** precoccinellene.

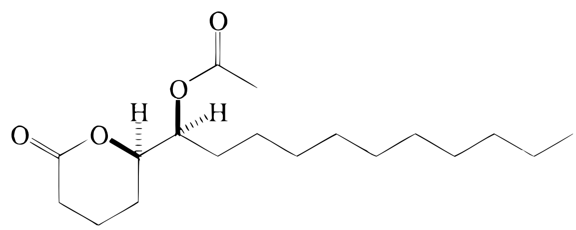

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Figure 9. Oviposition pheromone of *Culex quinquefasciatus*.

volatiles from this material located active compounds subsequently identified as phenol and indole. Olfactory cells for 3-methyl indole were also found, and the pure compound significantly increased oviposition in laboratory and field assays.

The *Culex* oviposition pheromone can be synthesised via a Baeyer-Villiger reaction[54] but for exploitation in resource-poor regions, a cheaper source of the material was considered necessary. A number of plants, including the summer cypress, *Kochia scoparia* (L) Schrad, contain significant amounts of the precursor 5-hexadecenoic acid. Fatty acid components of *K. scoparia*, including 5-hexadecenoic acid, were treated with a catalytic (and recyclable) amount of osmium tetroxide to afford the erythro-5,6-dihydroxydecanoic acid; simultaneous cyclisation to the δ-lactone and acetylation with acetic anhydride yielded the pheromone and its inactive enantiomer.[55] Production of this pheromone via a renewable plant resource represents an important development in sustainable and cheap pheromone production.

2.3.2 Cattle flies

Flies attacking cattle can lead to significant economic losses through disease incidence and reduced growth and milk production. Current control measures rely heavily on pesticides, and build-up of resistance and public concern about use of such materials in urban situations has increased pressure to investigate alternative methods of control based on the use of semiochemicals. Host location by haematophagous dipterans relies largely on volatile semiochemicals released by the body of the host and from by-products such as manure and urine. Studies on fly distribution within a cattle herd have revealed that some animals are more susceptible than others.[56,57] Field studies also showed that, by moving susceptible and less susceptible cattle between two herds, it was possible to redistribute the fly population within both. Volatile chemicals released from the body and urine of both types of cattle were isolated by air entrainment and using the GC-EAG technique, electrophysiologically active compounds were located and identified. In all, 26 compounds showed EAG activity, 18 coming from the body of the cattle and eight from the urine. Field trials are still under way to evaluate the effects that blends of such chemicals may have in redistributing the fly population in a push-pull context.

3 DEVELOPMENT OF THE SDDS

An initial demonstration of the stimulo-deterrent or push-pull strategy in the field, combining the principles of protecting the harvestable crop and aggregating the pests in a specific area, was achieved with the pea and bean weevil, *S lineatus*.[58] The 'pull' component was the aggregation pheromone of the weevil, identified previously as the 1,3-diketone **9** (Fig 7). The 'push' component involved a commercially available antifeedant based on an extract from the Indian neem tree, *Azadirachta indica* A. Juss. Although there are many claims in the literature for the effectiveness of neem extracts, in arable agriculture such materials do not compare favourably with conventional pesticides. However, against *S lineatus*, sufficient antifeedant activity was observed to allow further investigation within the push-pull strategy. In field trials comprising both 'push' and 'pull' components, significantly fewer *S lineatus* were found in the 'push' plots and more on the 'pull' plots, relative to the untreated controls. Although the 'push' plots were insufficiently protected for commercial agricultural systems, these trials nonetheless demonstrated the potential of such a strategy.

In a collaborative programme with ICIPE, funded by the Gatsby Charitable Trust, a novel crop-protection strategy based on the push-pull system has been developed for subsistence farmers in East Africa. Maize, *Zea mays* L., and sorghum, *Sorghum bicolor* (L.) Moench, provide staple food for millions of people in Africa, and lepidopterous stem borers such as *Chilo partellus* Swinh and *Busseola fusca* Full are major pests of these crops, often causing devastating yield losses. Field trials in Kenya showed that the forage grasses *Pennisetum purpureum* Schumach. (Napier grass) and *Sorghum sudanense* (Piper) Stapf (Sudan grass) attracted greater oviposition by stem borers than cultivated maize, whilst the non-host forage plants *Melinis minutiflora* Beauv. (molasses grass) and *Desmodium uncinatum* (Jacq) DC (silver leaf) repelled gravid female borers.[59] Push-pull trials, using the attractive plants as trap crops and the repellent plants as intercrops, reduced stem borer attack, resulting in a significant increase in yield.[23]

The semiochemicals mediating interactions between cereals, stem borers and their parasitoids have been identified using techniques of air entrainment, coupled GC-electrophysiology and MS, together with behavioural assays. Of the host plant semiochemicals identified, eugenol significantly enhanced oviposition, whilst α-terpinolene and β-caryophyllene, derived from the non-host molasses grass, deterred oviposition. In addition, the nonatriene **3** (Fig 4), produced naturally by molasses grass, attracted the larval parasitoid *Cotesia sesamiae* Cameron, and increased levels of parasitism were observed in the field using the maize/molasses grass intercropping system. This compound is also produced by herbivore-damaged plants, and is implicated more widely as a cue for stimulating predation and parasitism.[23]

For structure confirmation studies on tentatively identified compounds, and for behavioural assays to determine the ratios and concentrations eliciting optimum activity from the target organism, samples of authenticated material are required. In addition, larger quantities are necessary for field trials to assess

activity under natural conditions. It is therefore important, if strategies for use of semiochemicals in the field and in commercial situations are to be developed, to establish efficient synthetic routes for large-scale production, or to identify sources from which the materials are released naturally or can be extracted. The process can be exemplified for the aphid sex pheromones. For initial studies, the nepetalactone **6** (Fig 6) was extracted from the catmint plant *Nepeta cataria* L. and chemically reduced to afford the nepetalactol **7** (Fig 6). To produce these components synthetically on a larger scale, a method was adapted from work by Schreiber[60] to allow preparation of multi-gram quantities.[32] The route employs commercially available (*S*)-citronellol, which fixes the necessary stereochemistry in the product. Various levels of enantiomeric purity are available, 99% and 95% (*S*), 98% (*R*) and racemic, which has facilitated preparation of a range of nepetalactones and nepetalactols and their enantiomers. Studies using these compounds have demonstrated the effects of varying the stereochemical purity of sex pheromone components on attractiveness to aphids[35] and their natural enemies, including parasitoids.

4 FUTURE PROSPECTS

Prospects for extending the development of semiochemically based crop protection strategies are dependent on an increased understanding of the dynamics of the processes involved. This is particularly relevant in interactions at higher trophic levels, not only with the parasitoids and predators of pests, but also with insects such as hyperparasitoids which adversely affect populations of these beneficial organisms. Deeper insights into how host plants and unsuitable plants are identified by phytophagous insects will ensue from the discovery of the specificity of the underlying sensory processes. Semiochemicals, and the strategies for their use, must be integrated with other forms of pest control, involving pathogens, natural enemies and, of course, aspects of resistance conferred by plant breeding and by techniques based on recombinant DNA.

As new techniques in molecular biology have emerged, tremendous efforts have been made to find ways of using these techniques to improve crop resistance to pests and a great deal has been achieved in engineering genes which give rise to insecticidal proteinaceous materials. However, the possibility of modifying pathways leading to semiochemical production was also predicted. Although whole pathways were not envisaged initially as being the targets, it was expected that by opportunistic transformation, existing pathways to secondary metabolites could be modified to produce more useful semiochemicals. Some specific pathways have been chosen but without, as yet, practical realisation of their potential. Nonetheless, the approach to modifying secondary metabolism pathways to give improved products has been demonstrated and, with this knowledge of success and the acknowledgement of limitations, further refinement in targets can be considered.

It is also proving possible to exploit production of semiochemicals by plants as a bridging point in the biotechnological production of semiochemicals by molecular biological techniques. A great many studies are in progress, and an expansion of the subject of physiologically active compounds from plant secondary metabolism is currently taking place. The success of aphid sex pheromone studies in demonstrating the considerable potential for the manipulation of aphid parasitoids has led to the initiation of a collaborative project involving IACR-Rothamsted, English Hop Products Ltd, Agrisense BCS Ltd and the Richard Wood Hop Partnership (LINK project, UK Ministry of Agriculture, Fisheries and Food). These collaborators are working together to develop pilot systems for sex pheromone production from *N cataria*, leading ultimately to the commercialisation of aphid sex pheromones for the manipulation of beneficial insects.

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